tion during the earlier months of the experiment may be largely the resultant of other interrelationships. Consider this possibility in detail.

Let r_{ye_1}, r_{ye_2}, r_{ye_3}, . . . $r_{ye_{12}}$ be the correlations between percent yellow in the twelfth month of the contest and egg production in the first, second, third . . . twelfth months respectively. Further let $r_{e_{12}e_1}$, $r_{e_{12}e_2}$, $r_{e_{12}e_3}$ . . . $r_{e_{12}e_{11}}$ be the correlations between October and November, October and December, October and January . . . October and September egg productions. These constants have been shown to be positive throughout, indicating that birds excelling in egg production in October gave on an average higher productions in every other month of the year.

The application of the well known partial correlation formula for one variable, e_{12}, constant results in very material reductions in the values of r_{ye_1}, r_{ye_2}, r_{ye_3}, . . . $r_{ye_{11}}$. Thus the values of r_{ye_1}, r_{ye_2}, r_{ye_3}, . . . $r_{ye_{11}}$ must be in large part the resultants of $r_{e_{12}e_1}$ and $r_{ye_{12}}$, $r_{e_{12}e_2}$ and $r_{ye_{12}}$, $r_{e_{12}e_3}$ and $r_{ye_{12}}$ . . . $r_{e_{12}e_{11}}$ and $r_{ye_{12}}$.

A discussion of the biological and biochemical literature, and a detailed statistical treatment of the data are appearing in *Genetics*, 1917.

[1] Ear lobe color has been measured in units of 5% range by means of the color top. Only yellow and white sectors were used.

[2] All birds entered the international Egg Laying Competition at Storrs, Connecticut, in November of their pullet year, and remained until the end of October of the following year. Pigment determinations were made near the end of October.

[3] When the constants for the comparable months in the two years are considered in comparison with their probable errors, there is not a single difference which can be considered significant.

[4] The biological inference to be drawn from this result would seem to be that the egg production of a recent period influences very profoundly the concentration of yellow pigment, so that there is a very rapid decrease in yellow pigment for each additional egg laid up to a certain point, beyond which the body pigment is relatively little reduced by extra egg production. Thus for October, the change in pigmentation is to be described by a curve, not by the slope of a straight line. The change in pigmentation is not proportional to egg production, but at first is very rapid and then falls off.

[5] In collecting these data a bird which laid on the day the pigment determination was made or on a later day within the month was considered to be laying, and was recorded in the zero class, *i. e.*, no days since laying. If she laid on the day before the record was taken but not later she is recorded as one day since laying, and so on.

VARIABILITY OF GERM CELLS OF SEA URCHINS

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As a basis for an understanding of the changes in aging germ cells, it was necessary first to ascertain the normal variability, i.e., the varia-

bility of eggs and sperm removed immediately after receiving the freshly collected sea urchins. For this purpose three different species of sea urchin were studied, namely, *Toxopneustes* and *Hipponoë*, of the shallow tropical waters of the Dry Tortugas, Florida, and *Arbacia* of the deeper colder waters of Woods Hole, Mass.

To obtain optimum conditions as nearly uniform as possible, preliminary experiments were made with each species. These experiments established the optimum concentration of fresh eggs and of fresh sperm for a given surface and volume of filtered sea water. For example about 800 eggs in 10 cc. of sea water, in Syracuse dishes, with 0.05% sperm, with care exercised to avoid evaporation, gave optimum results with *Toxopneustes* and *Arbacia*. For *Hipponoë* the same conditions sufficed except that much greater concentration of sperm was needed.

Having ascertained the optimum and constant conditions it was found that the remaining variability was in large measure a function of the particular male and female used. If cleavage be used as a measure of variability and if the eggs of a female be fertilized by different males, widely varying percentages of cleavage occurred. The female whose eggs gave the highest percent of cleavage by one male, usually gave the highest percent by other males, though the absolute percentage differed considerably. And *vice versa* a low cleavage female usually gave low cleavage by other males. A few experiments are cited to show some of the details (table 1).

With these facts in mind a detailed study of certain variations were made as follows: (a) variations in size and shape of the eggs; (b) variation in the jelly layer of eggs; (c) variation in membrane formation; (d) variation in cleavage.

(a) Eggs of a considerable number of females of each species were carefully measured. The eggs of some females varied little from the norm, others varied much, usually by enlargement. One set of readings of two females collected and prepared at the same time is given below.

Ocular readings of diameter of eggs

♀	21	20.5	20	19.5	19	18.5
1	0	0	0	0	33%	67%
2	8%	24%	48%	24%	0	0

Female number 1, varied but little from the norm (19); female number 2 showed considerable enlargement, and variability. Eggs normal or nearly normal in size tended to be all or nearly all globular, eggs showing considerable variation tended to be elliptical.

(b) The number of eggs with intact jelly layer also varied in the different females. Some females contained eggs, nearly 100% of which possessed the jelly layer, others as low as 40%, etc. Such reduction was correlated with an increase in size, and an increase in the number of elliptical eggs.

TABLE 1

THE VARIATION IN CLEAVAGE WITH DIFFERENT MALES OR FEMALES

Toxopneustes

NUMBER OF		PER CENT CLEAVAGE	NUMBER OF		PER CENT CLEAVAGE
Females	Males		Females	Males	
1	1	34	1	1	79
2	1	71	2	1	83
3	1	16	3	1	98
			4	1	99
1	2	24			
2	2	24	1	2	65
3	2	9	2	2	60
			3	2	98
1	3	20	4	2	100
2	3	95			
3	3	33	1	3	61
			2	3	48
			3	3	85
			4	3	81

Arbacia

Females	Males		Females	Males	
1	1	34	1	1	99
2	1	71	2	1	25
3	1	16	3	1	57
1	2	24	1	2	83
2	2	24	2	2	64
3	2	9	3	2	30
1	3	20	1	3	98
2	3	95	2	3	93
3	3	33	3	3	50

TABLE 2

TO ILLUSTRATE THE RANGE OF VARIABILITY IN RATE OF MEMBRANE FORMATION IN FRESH GERM CELLS

NUMBER OF		MINUTES REQUIRED FOR FIRST MEMBRANE TO APPEAR
Females	Males	
1	1	6
2	1	4
3	1	4
1	1	2
2	1	$1\frac{1}{2}$
3	1	$1\frac{1}{2}$
4	1	$1\frac{1}{2}$
1	2	$\times$
2	1	$2\frac{1}{2}$
3	1	$1\frac{3}{4}$
4	1	1
1	1	$\times$
2	1	$\times$
3	1	$2\frac{1}{2}$
4	1	$2\frac{1}{2}$
5	1	$\times$
6	1	$\times$
7	1	$2\frac{1}{2}$

$\times$ Signifies that no membrane formed in ten minutes.

(c) The rate of membrane formation also varied in different females. In some, the eggs formed membranes within two minutes, in others three to ten minutes, in others not at all. Rapid membrane formation was correlated with slight variability in size, globular shape of eggs, and high percent of jelly layers; slow membrane formation with the reverse conditions. A few examples are cited to illustrate this type of variation, in the eggs of different females fertilized by the same males (table 2).

(d) The rate of early cleavage (first and second) and the total cleavage showed even greater variations, from complete or almost complete sterility to 100%. High percent cleavage was correlated with little variation in size, globular shape, high percent jelly layer, and rapid membrane formation. The reverse conditions were associated with low cleavage. In these ways one may separate the so-called 'good' from 'bad' eggs, which morphologically are indistinguishable. A single example of 'good' and 'bad' females are given below:

SPECIES	NUM-BER OF MALE	NUMBER OF FEMALE							MAXI-MUM DIFFER-ENCE	TIME CON-STANT
		1	2	3	4	5	6	7		
Toxopneustes.....	1	99	83	98	99				16%	2 hrs.
	2	81	76	18	14	40			72	
Hipponoë........	1	92	97	83					14	
	2	90	54	59	47	16			74	
Arbacia.........	1	100	100						0	
	2	99	98	97	97	98	99	99	7	
	3	81	58	1	77	91			90	

The amazingly large variability in these fresh germ cells finds its explanation in the following facts: (1) There is a primary small variation in fresh eggs of any female. (2) Eggs do not all ripen at the same time but at different intervals, within the body of the female. (3) Injurious changes occur in the eggs from the moment they ripen. (4) The time of elapsing between maturation and removal from the body may be different for different females, and therefore the intensity of the changes will be different for different females. (5) Sea water is also slightly injurious to ripe eggs, and the already different physiologic condition of the eggs is further heightened by the differential effect of the sea water.

Eggs removed from different females at the same time though of the same chronologic age are nevertheless rarely in the same physiologic condition. In order to determine their physiologic state and therefore, in order to separate the eggs according to their physiologic condition, it becomes necessary to ascertain in the manner just described the degree of variation of size, of jelly, and better still of membrane formation, and still better of cleavage, as well as by other means.

It becomes highly probable in view of these facts that the varying behavior of the eggs in the experiments of J. Loeb, F. Lillie, R. Lillie, Wasteneys and others was due in large part to a variation in the physi-

ologic conditions of the eggs which these investigators used. These variations have now been adequately described and measured, and correlated with physiologic condition of the eggs. For experimental work it is absolutely necessary to determine in advance the exact physiologic condition of the eggs, and to use only such, as are nearly in the same condition. We may then hope to obtain more constant and predictable results.

A full account of these experiments will appear in the forthcoming volume of the Researches of the Marine Biological Laboratory of the Carnegie Institution of Washington.

TRANSPLANTATION OF LIMBS

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The specificity of the tissue of the limb bud in the amphibian embryo has been clearly shown by Braus,[1] who found that when transplanted to any part of the body it would develop into a normal appendage. Since then evidence has accumulated[2] to show that the limb rudiment, and more especially its mesoderm, constitutes an equipotential system.[3] Legs are, nevertheless, rights or lefts and, having no plane or axis of symmetry, the leg of one side of the body cannot be superimposed upon that of the other. In the early embryonic condition, however, there is no visible evidence of laterality, and the question arises when and how this property is determined. Experiments made during the past year have rendered it possible to state more simply than before[4] the rules that govern its determination.

All of the experiments here considered were made with the fore limb of Amblystoma punctatum under precautions necessary to prevent regeneration from the host.[2] In grafting the limb buds three different circumstances relating to their position in the embryo were taken into account—location, laterality and orientation (fig. 1). First, the limb buds were placed either in their natural location in another embryo after removal of the normal bud (orthotopic transplantation) or else in some other region of the body, preferably on the flank of the embryo between the normal fore and hind limbs (heterotopic transplantation). Secondly, some were grafted on the same side of the body as that from which they were taken (homopleural) and others on the opposite side (heteropleural). Thirdly, they were placed either in upright position, with the dorsal border of the transplanted disc corre-